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ABOUT THE AUTHOR

Dr. Josef Warkany is Professor of Research Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio. He conducts his research activities at the Children's Hospital Research Foundation, Cincinnati, Ohio. Dr. Warkany has been a pioneer in the induction of a large variety of congenital malformations in experimental animals by nutritional means.

Born in Austria, Dr. Warkany received his elementary education there and obtained his medical degree from the University of Vienna in 1926. In addition to his outstanding research contributions in experimental teratology, he was trained as a Pediatrician and is a member of the American Board of Pediatrics, the American Pediatric Society and the Society for Pediatric Research.

In recognition of his outstanding contributions, he was awarded the Mead Johnson Award by the Academy of Pediatrics in 1943 and the Borden Award in Pediatrics in 1950.

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Nutritional Experiments as an Instrument of Teratologic Research in Mammals

Josef Warkany, M.D.

INTRODUCTION

It has been possible for over 100 years to produce monstrosities in chicks by various environmental procedures, and for many decades a variety of anatomical defects have been produced experimentally in amphibia and fishes. Ova and embryos of animals of lower classes are easily accessible and modification of their development is possible without difficulty. Experimental embryologists have long made use of such ova to study fundamental processes of embryogenesis.

Ova of mammals which develop in the maternal uterus are well protected against adverse influences of the outside world. They are not easily observed and reached for experimentation. Yet there existed a great need for such experiments, since anomalies induced in animals of lower classes are not always comparable with mammalian or human malformations. In addition, it seems possible that disturbances of reproductive processes limited to mammals can lead to malformations, and such disturbances cannot be studied in lower classes.

It was generally admitted that malformations of the offspring of animals whose ova develop outside the maternal body could be due to environmental injuries. However, many students of human malformations thought that the protection afforded by the maternal uterus precluded exogenous damages to the human embryo. They thought that most, if not all, congenital malformations in mammals were predetermined in the germ cells or in the zygote, the cell formed by the union of the sperm and the ovum. In fact, there was and is much proof that many malformations are genetically determined at such early stages of development and that they make their appearance in spite of the most favorable environmental conditions.

At the turn of the century when the Mendelian laws were rediscovered, it was soon found that certain congenital defects behaved like normal Mendelian characters. Thus, some defects were transmitted as dominant or recessive traits. Such anomalies were often seen repeatedly in families or kinships and were recognized as inherited traits. It was also established that sporadic cases appearing only once in a family or pedigree could be determined genetically. Hence, it was soon believed and asserted that all congenital malformations are hereditary. It was known that occasionally the malformation of a child could be attributed to mechanical interference with the growing fetus and that heavy x-irradiation of a pregnant woman could result in deformity of her child. Such cases, however, were rare and exceptional. Thus, internal, symmetrical and systemic congenital malformations were frequently attributed to genetic factors while exogenous factors were not considered. The need to demonstrate that subtle, pathologic factors could modify the normal development of mammalian embryos remained.

EARLY TERATOLOGIC EXPERIMENTS WITH MAMMALS

Early in the 1930's, Hale gave the impetus needed for promoting the study of nutritional factors affecting the normal development of mammalian embryos. Prior to that time a number of observations had been made which suggested that blindness, weakness and deformity of newborn animals are sometimes due to poor nourishment of the mother. But it was not until Hale's experiment,[1] begun in 1933, that maternal dietary deficiencies, under certain experimental conditions, conclusively were shown to interfere with mammalian embryonic development and to induce congenital malformations in the young.

Hale applied his experimental procedure to pigs of different breeds. A total of 59 pigs were obtained from vitamin A-deficient gilts showing a variety of congenital malformations. He demonstrated that microphthalmus and anophthalmus were induced in pigs when the mother was depleted in vitamin A during the first month of gestation. Supplements of cod liver oil given after this period did not prevent ocular anomalies but did protect the fetuses against complete destruction. He also showed (1) that the males used in the experiments produced normal offspring when bred to gilts fed an adequate diet and (2) that when a male with induced congenital blindness was crossed to a normal, unrelated gilt, to its dam or to a blind sister litter-mate, it farrowed normal young if the mother was on an adequate diet. These experiments apparently have not been repeated nor extended, since a large and expensive animal such as the pig is not suited for serial and controlled studies.

Hale mentioned in his report that congenital blindness occurred in the offspring of vitamin A-deficient rats. However, nearly a decade passed before adequate experimental techniques were developed for large-scale, teratologic experiments with small-sized, fast-reproducing mammals such as rats and mice. In the meantime, a few reports of abnormalities in the offspring of rats reared on deficient diets were published. For instance, in 1941 Anderson[2] observed diaphragmatic hernia in some offspring of vitamin A-deficient female rats.

TECHNIQUES DEVELOPED FOR LARGE-SCALE TERATOLOGIC EXPERIMENTS

Between 1940 and 1944 Warkany and Nelson[3, 4] found that rats reared on a rachitogenic diet supplemented with vitamin D matured late and bred late. When the females finally became pregnant, many resorbed their young. Of the offspring carried to term, 37% showed many skeletal abnormalities. The multiple skeletal malformations included: shortening or absence of the tibia, fibula, radius, ulna, or the bones of the hands and hind feet; shortening of the mandible; cleft palate; fusion of the ribs and various deformities of other bones. These abnormalities were absent in the offspring of control animals of the same hybrid strain when the females were reared on an adequate stock diet or when liver was added to the experimental diet. With this procedure, hundreds of defective offspring could be obtained. Thus, nutritional teratologic experiments on a large scale now became possible.

ADVANTAGES OF SMALL MAMMALS FOR TERATOLOGIC RESEARCH

Working with a small laboratory animal had the advantage that histologic serial sections and morphogenetic studies could be conducted.

Histologic examination of the bones of the newborn rats of mothers fed a rachitogenic diet plus vitamin D showed: (1) that ossification was delayed; (2) that the orderly relationship between the calcifying cartilage and the osseous parts was disturbed; (3) that cartilage persisted in areas where ossification should have taken place, and (4) that the cartilage itself was abnormal. Morphogenetic studies of the embryos revealed that anomalies, such as syndactylism or reduction in the number of fingers, were recognizable in the mesenchymal stage of the skeleton. Thus, the deficiency did not primarily cause abnormal calcification or ossification; it was already expressed in the precursors of bone, namely, cartilage and mesenchyme.

Experiments were undertaken to ascertain how late in pregnancy the malformations could be prevented by adding liver to the deficient diet. It was found that prevention was always possible before the 13th day of gestation but not after that day. This is approximately the time of differentiation of the membranous skeleton into the structures which later become the individual bones of the definitive, appendicular skeleton. It was concluded that liver contained a nutritional factor needed for the development of the skeleton, particularly between the 13th and 14th days of gestation, when the rat embryo grows rapidly and the future bones are laid down in their mesenchymal form.

Next, the preventive nutritional factor in liver was sought. Many supplements such as liver ash, iodine, manganese, casein, cod liver oil and wheat germ oil were not preventive.

Since liver contains many vitamins of the B-complex, attention was directed to the vitamins of this group as they became available in crystalline form. Thiamine, niacin, riboflavin, pantothenate and pyridoxine were employed as supplements in various combinations. It was established that only those supplements which contained riboflavin alone or in combinations were preventive. That riboflavin was actually the preventive factor for these skeletal anomalies was further demonstrated by the use of a purified diet in which the vitamin B complex was represented by crystalline substances. The malformations appeared only when riboflavin was omitted from the maternal diet while deformed offspring were not obtained when the diet contained riboflavin. That riboflavin was essential for the proper differentation of the skeleton was a somewhat new and startling conclusion at that time.[5]

THE USE OF PURIFIED DIETS IN MAMMALIAN TERATOLOGIC RESEARCH

The availability of a purified diet from which single vitamins could be omitted led to further investigation in nutritional teratologic research.

Warkany and Schraffenberger[6] reared female rats on a preparatory diet of low vitamin A content and supplemented periodically by small doses of carotene. The amounts of added carotene were sufficient to permit slow growth and maturation but not storage of the vitamin. When these rats reached sexual maturity they were given a purified diet free of vitamin A and were bred. It was noticed that some of their young had "open eyes" at birth, an anomaly in newborn rats. Histologic sections showed that the most frequent ocular anomaly was development of connective tissue masses between the hyaloid vessels in place of the vitreous. In addition, coloboma, retinal eversion and rosettes, and anomalies of the iris, of the cornea and of the lids were found. Further investigations revealed that many of the young had diaphragmatic hernia, as observed previously by Anderson, as well as a variety of urogenital and cardiovascular malformations. The bones of these young rats did not show malformations of the pattern characteristic of the offspring from the riboflavin-deficient mothers found in the earlier experiment. Female rats reared and bred on the same vitamin A-low diets supplemented with vitamin A had normal offspring. An increase in the amount of carotene of the preparatory diet improved the fertility of the females, but reduced the incidence of the abnormal young. Conversely, reduction of the carotene supplements reduced fertility and the percentage of abnormal young increased.[7]

EFFECTS OF VITAMIN A DEFICIENCY

Maternal vitamin A deficiency induced congenital anomalies of the heart and of the great vessels which could be studied anatomically, histologically and embryologically. There were aortic arch anomalies, interventricular septal defects and retardation of myocardial development. Many of the cardiovascular anomalies observed in the experimental animals closely simulated malformations observed in man.[8] Also with the help of this method, numerous embryos and newborn rats with hypoplastic or horseshoe kidneys, pseudo-hermaphroditism or hypospadias were obtained.

An observation of general interest was made on the epithelia of the genitourinary tract. In general the cardinal sign of vitamin A deficiency — namely, the appearance of stratified keratinized epithelium — was absent in the fetuses and young of vitamin A-deficient mothers, although many malformations attributable to vitamin A deficiency were present. However, keratinizing metaplasia was found in the epithelia of structures derived from the urogenital sinus in fetuses over 18 days gestational age. This observation suggests that embryonic tissues show a very different response to vitamin A deficiency from that observed in tissues of older fetuses and of the organism after birth.[9]

Although there was considerable variation of the malformations of the young of vitamin A-deficient mothers, the anomalies conformed to a certain pattern. Of the abnormal offspring, over 90% had both ocular and urogenital anomalies, 58% had diaphragmatic hernia, while only 17% had cardiovascular malformations. Thus, a syndrome could be recognized in which some anomalies were predominant and nearly always present while others were rarely present.

The vitamin A deficiency syndrome in the offspring could be modified.[10] This was done by interrupting the maternal dietary deficiency by administration of large amounts of vitamin A on certain days of gestation. It was found that the percentage of abnormal young became smaller the earlier the supplement was given. But it was also found that the prevalence of certain anomalies changed according to the date of vitamin A administration. For example, the ocular and urogenital anomalies were markedly reduced in number if vitamin A was administered on the 13th day of gestation, but the incidence of cardiovascular anomalies was increased. It seems that vitamin A deficiency damages the heart and the great vessels before the 13th day while the interference with the ocular structures occurs later. Thus, the vitamin supplement could prevent the anomalies of the eye but not those of the cardiovascular system, whereby the pattern of vitamin A deficiency was changed.

LATER EXPERIMENTS AND IMPROVED METHODS

These early experiments demonstrated that the development of the mammalian embryo, like that of embryos of lower classes, could be modified by environmental disturbances. They also showed that systematic embryological studies could be undertaken in small laboratory animals with methods based on maternal dietary deficiencies.

Some of the early teratologic experiments with rats were repeated and confirmed in other laboratories.[11] Later other teratogenic deficiencies were recognized.

Hogan et al.[12] found that a maternal diet deficient in folic acid and vitamin B_{12} induced a high incidence of hydrocephalus in infantile rats whereas hydrocephalus was seen associated with vitamin A deficiency in rabbits.[13] Lefebvres-Boisselot[14] found exencephaly and ocular defects in rat fetuses of mothers given a pantothenic acid deficient diet beginning 3 to 10 days before mating. Malformations could also be induced in rats by maternal vitamin E deficiency if resorption of the fetuses was prevented by giving supplements of vitamin E during early organogenetic periods.[15] Other examples of congenital malformations attributable to maternal dietary deficiencies can be found in review articles on the subject.[11, 6]

It must be admitted that the early methods based on maternal dietary deficiencies were laborious and capricious. They proved effective only in the hands of dedicated workers. The induction of malformations in embryos requires creation of borderline deficiencies which injure the embryo without completely destroying it. A delicate balance must be maintained, for instance, between the depletion and the amounts of carotene fed to the mother to maintain her life and that of her fetuses. The depletion periods required before mating had to be found empirically. Thus, these preliminary periods lasted sometimes many weeks or even months. At the end of such preparatory periods many females had become sterile and were lost for the experiment. In early studies the experimental animals were not delivered by Cesarean section but were allowed to bear their young spontaneously. This was necessary to secure as many consecutive litters as possible in

order to demonstrate that the incidence of abnormal young increased with increasing depletion or that the same male and female rats could produce abnormal or normal young according to the diet fed. As depleted rats are notoriously cannibalistic, the females had to be watched closely by day and night as the time of parturition approached. If not done, one often found that the animal had delivered but the precious offspring had disappeared. In the early years of our investigations my co-workers often took expectant rat mothers home to be near them at the hour of confinement. Later when the numbers of our obstetrical patients became very large, three work shifts were instituted to insure observation around the clock. It is understandable why this type of experiment did not become very popular and remained confined to few laboratories.

ROLE OF SPECIFIC ANTIMETABOLITES IN TERATOLOGIC RESEARCH

Nutritional teratologic methods were much simplified and improved when specific antimetabolites became available.[17] The use of antimetabolites made possible in many instances the rapid production of acute dietary deficiencies. In our original experiments, for instance, female rats had to be depleted of riboflavin for about six weeks before mating to induce a riboflavin deficiency during gestation and to obtain abnormal offspring. Riboflavin deprivation limited only to the time of gestation was insufficient; it did not induce abnormal young. When Nelson et al.[18] added the antimetabolite, galactoflavin, to a riboflavin deficient diet, the deficiency could be created almost at once.

Antimetabolites made it possible to omit the long preparatory period, to limit the experiment to the time of gestation and to double the yield of abnormal young. The skeletal malformations induced by the use of galactoflavin were, in general, very similar to those induced in the earlier experiments by riboflavin deficiency alone. However, the addition of galactoflavin did change the syndrome since soft tissue malformations occurred in more than one-third of the abnormal offspring. There were, in addition to the skeletal changes, cardiovascular, urogenital, ocular and cerebral malformations, and the results were clearly influenced by the time the antimetabolite was administered. The specificity of the method was demonstrated by the fact that riboflavin supplements prevented the malformations.

Folic acid (pteroylglutamic acid) deficiency was developed into an excellent teratogenic method. Giroud and Lefebvres-Boisselot,[19] adding 5% succinylsulfathiazole (sulfasuxidine) as an intestinal antibiotic to a maternal diet deficient in folic acid, induced in rats ocular and cerebral defects, cleft lip and palate, ectopia cordis, gastroschisis and other malformations. Using the antagonist, x-methylfolic acid, Nelson et al.[20] created acute folic acid deficiencies in pregnant rats. The results varied according to the time the drug was administered. If the deficiency was instituted 11 days after breeding and continued throughout pregnancy, 95% of the animals littered young with multiple congenital abnormalities.

In later experiments Nelson et al., by creating short periods of folic acid deficiencies during two or three days of gestation, obtained a variety of malformations in the offspring. Cleft lip and palate, syndactylism, polydactylism, urogenital, cardiovascular and cerebral anomalies could be induced with differing frequency by short and precise experimentation. Cleft palate was obtained, for instance, in 100% of the young when the period of deficiency extended from day 10 to 13; harelip (usually with cleft palate) was seen in 86% after a period of deprivation from day 9 to 11. One of the interesting observations in these studies was that the mothers receiving the folic acid-deficient diet gained weight throughout the experimental period and were in good condition, regardless of the deleterious effects upon the fetuses. This probably explains the high yield of abnormal young obtained by this rapid treatment. It makes possible the survival to term of a large number of deformed fetuses in the uterus of mothers who remain apparently in good health.

The use of antimetabolites also proved effective in the creation of pantothenic acid, amino acid, or nucleic acid deficiencies. For details the reader is referred to comprehensive review articles [11, 16, 17] and to the original publications.

After this discussion of dietary deficiencies as teratogenic methods it may be somewhat surprising to learn that a maternal hypervitaminosis may also lead to the birth of abnormal offspring.

Cohlan[21] discovered that huge doses of vitamin A administered to pregnant rats during organogenesis induce malformations in many of the young. Exencephaly, hydrocephalus, spina bifida, ocular defects, cleft palate and other skeletal malformations occurred as the result of this treatment. It is possible that vitamin A acts in such experiments like other toxic substances known to be teratogenic. There can be no doubt that large amounts of vitamin A, regardless of their mode of action, are effective in teratogenesis, as Cohlan's observations have been amply confirmed by other investigators.

COMBINATION OF NUTRITIONAL AND GENETIC STUDIES

Some remarks must be made at this point about the animals used in these experiments of mammalian teratology.

Most of the work mentioned in this paper was done with hybrid rats of commercial stocks or with rats bred and reared for many years in scientific institutions. As a rule the results of the teratogenic procedures were the same irrespective of the origin of the rats. If minor differences were observed in different laboratories, they were sometimes attributed to strain differences, but genetic studies were not made in these rat experiments.

It seemed of interest to combine the observations of nutritional effects with genetic studies. We attempted repeatedly to reproduce the results obtained in rat experiments in mice of inbred strains.

It was known that different strains of inbred mice have differing tendencies to spontaneous congenital malformations. It appeared desirable to ascertain teratogenic effects of dietary deficiencies upon such inbred strains. In our early

attempts we failed to induce in mice congenital malformations by simple maternal dietary depletion. The necessary borderline deficiencies could not be produced easily in mice since the females became either sterile or gave birth to normal young.

Runner and Miller[22] made some interesting teratologic observations on the young of mouse strain 129. After females of that strain were fasted for 24 hours during the 9th day of pregnancy, exencephaly, vertebral anomalies and fused ribs were found in 28% of the offspring. This was surprising. In the past numerous studies of the effects of inanition and unspecific malnutrition upon reproduction had not resulted in structural changes of the fetus.[23] However, owing to the genetic make-up of mice of strain 129, temporary maternal starvation led to congenital malformations of the young. Similar malformations were observed by Runner[24] in the young of the same strain when on the 9th day of pregnancy he fed a folic acid-deficient diet supplemented by x-methylfolic acid. Thus, it was shown that by use of an antimetabolite a teratogenic nutritional deficiency could be created in mice where simple depletion had failed.

In our laboratory[25] riboflavin deficiency was produced in four inbred strains of mice. It was done with the help of galactoflavin and a modification of the procedure successfully used with rats by Nelson et al.[18] A large variety of malformations was obtained in the young when the diet was fed to female mice from the 10th to the 14th days of gestation. The mice were of the inbred A/Jax, DBA, 129 and C_{57}BL strains. Skeletal as well as soft tissue malformations were found. Some of these malformations resembled those produced by similar methods in rats while others differed from them. There were also differences in the malformations in the four strains of mice. Atresia of the esophagus, for instance, was induced by this method in about 89% of the young of strains A/Jax and DBA, but in only 2% of the C_{57}BL offspring. Brain anomalies were also more frequent in the young of the A and DBA strains than in the C_{57}BL and 129 offspring. Patterns of malformations were more or less specific for each strain, demonstrating the influence of genetic factors upon the malformations.

PROBLEMS OF INTERPRETATION

Many nutritional methods are now available for teratologic research. While it has been demonstrated beyond doubt that maternal dietary deficiencies can bring about, under strict experimental conditions, severe structural anomalies in mammalian embryos, it is much less certain how such experiments can be interpreted and applied to questions of human teratology.

It should be emphasized at the outset that the experiments cited do not prove that congenital malformations in man are due to maternal dietary deficiencies. There are many reasons why speculations about applications to human conditions should be received with caution. The experimentors acquainted with the details of the methods usually appreciate the differences between the experimental conditions and conditions of human reproduction.

In order to create teratogenic borderlines which injure the embryo without killing it, the depletion time must be regulated according to the ability of the maternal tissues to store or lose the substances of which the organism is to be depleted. If the depletion is too severe, the damaged embryos may be destroyed early, so that sterility ensues rather than delivery of malformed offspring. To salvage the young, the experimentor must sometimes supplement the diet at certain periods of gestation to avoid complete destruction of the damaged fetuses. In some experiments inhibitors or antimetabolites are given to achieve depletion. In human fetuses the period of intrauterine growth which follows the period of organogenesis is absolutely and relatively longer than that of rodents which probably reduces the chances for survival to term of deformed embryos.

Genetic and species differences must also be taken into consideration. It has been shown in some experiments that the outcome depends upon the genetic constitution of the species or strain selected. The fact that a teratogenic method produces malformations in mice does not permit the conclusion that it will have the same effect in rats. It is even more hazardous to apply uncritically results obtained in mice to human situations. Although deformed children are not rarely born to mothers whose diets during pregnancy had been inadequate, it is not justified to attribute such malformations to the maternal diet, since genetic and other non-dietary factors could have been at fault.

NUTRITIONAL FACTORS THAT INFLUENCE DEVELOPMENT OF THE HUMAN FETUS

In certain instances adverse nutritional conditions of the mother can impair the development of human fetuses. Thus endemic cretinism has been attributed to a maternal nutritional disturbance. Endemic cretinism is a syndrome of multiple congenital defects which can be found in isolated regions of the Alps, Pyrenees, Andes, and the Carpathian and Himalayan mountains. Endemic cretins are characterized by goiter, dwarfism, deafness, skeletal anomalies and mental deficiency. Such cretins are found in areas where goiter is endemic. There is little doubt that endemic goiter and cretinism are the result of adverse environmental conditions and they are usually attributed to maternal iodine deficiency. Although the etiology of endemic goiter[26] and cretinism is complex and not fully understood, these conditions disappear when improved diets or iodized salt are introduced into endemic areas.[27]

Antimetabolites that are teratogenic in animals may also be teratogenic in man. Deformed fetuses and children have been observed whose mothers had ingested aminopterin.[28, 29] Thus human embryos could probably react in similar ways to adverse nutritional conditions as animals, if the experimental conditions were simulated by circumstances of human reproduction. However, even in times of war and dietary deprivations an increase of congenital malformations has not been observed. It seems that women stop menstruating or become sterile if the deprivation is severe, or give birth to small but otherwise normal children.[30, 31] The delicate borderline conditions achieved in animal experiments are apparently

not easily simulated in human pregnancies. The fact that starvation in early pregnancy can cause malformations in the young of some inbred strains of mice should be kept in mind, since anorexia, vomiting, or lack of food could in rare instances cause comparable situations in women. However, experiments have shown that the effects of such early starvation become manifest only under certain genetic constellations. While it is worthwhile to direct attention to comparable situations in man, it must be emphasized that the mothers of most children seen in our hospitals and clinics because of congenital malformations did not encounter any obvious nutritional difficulties during pregnancy.

THE VALUES OF NUTRITIONAL TERATOLOGICAL RESEARCH

Although the nutritional experiments with animals may not be of immediate practical importance, they have yielded many results of considerable theoretical interest. They showed, for instance, that nutritional factors such as riboflavin, vitamin A, folic acid and others are needed for the differentiation of embryonic tissues revealing requirements which had not been recognized before. They demonstrated that adverse environmental conditions can cause congenital malformations in mammals. This is not a surprising result today, when it is generally accepted that maternal German measles can bring about congenital malformations in children. When the early investigators began work in nutritional teratology this was not known and experimental methods to produce congenital malformations were needed. The results indicated that adverse environmental conditions must be considered as possible causes of congenital malformations. While it was often assumed about twenty or thirty years ago that all congenital malformations are genetically determined and hereditary, it is now generally realized that subtle and subclinical environmental adversities must be considered as possible causative factors.

These results in no way speak against the importance of genetic factors in the etiology of congenital malformations. In fact, it seems likely that by recognition and exclusion of malformations of non-genetic origin, investigations of heredity malformations will be benefited and simplified.

POSSIBLE INTERRELATIONSHIPS BETWEEN DIETARY DEFICIENCIES AND MALFORMATIONS OF GENETIC ORIGIN

There exist some interesting relationships between malformations induced by maternal dietary deficiency and malformations of genetic origin.[32] While these two kinds of malformations differ completely both in their origin and in their importance for the species, they do show similarity in their morphology and possibly in their mechanisms of development. By maternal dietary deficiencies, types of congenital malformations can be induced which resemble morphologically malformations of genetic origin. For instance, cleft palate, syndactylism or microphthalmus may be genetic in origin; similar anomalies can be produced experimentally as non-hereditary imitations (phenocopies) by riboflavin, folic

acid or vitamin A deficiencies. Nutritional deficiencies like genes can induce syndromes of multiple anomalies. An abnormal gene may affect a variety of organs which in postnatal life seem to be unrelated to each other. Similarly, deficiencies in vitamin A or folic acid result in anomalies of the eye, of urogenital organs and of the cardiovascular system.

A resemblance exists between genetic defects and chronic continuous nutritional deficiencies as described in the early experiments, in that both conditions exist througout embryonic life and act only as the embryo reaches a stage in which a specific need becomes manifest.[5] In both cases the embryo develops against a permanently abnormal background. This fact is of great theoretical interest since it should caution against statements that the time factor is all-important for the occurrence and the type of malformations observed and produced. Such statements imply that one can, in the case of human malformations, retrospectively find the time of injury to the embryo by the knowledge of critical periods' of development. Such critical periods exist but they lead, in the presence of adverse genetic or chronic environmental conditions, to malformations without any special external events at the critical time. It is true that acute teratogenic agents such as x-rays, induced anoxia, antimetabolites and other toxic substances which attack the embryo only once and immediately after administration show differing effects according to the time they are applied. But continuous nutritional deficiencies, like genetic deficiencies, are chronic abnormal states which remain latent during some periods but become manifest in others when special demands are made by the embryo's metabolism.[33, 5] This is a point of some practical importance since there is now a widespread belief that one can consider external events as causative of congenital malformations if they happen to coincide with certain critical periods of embryonic development. This belief would be fallacious in the presence of chronically abnormal conditions.

In recent years many teratogenic methods have been developed which supplement early nutritional studies. In a recent review[11] over fifty metabolic procedures for experimental production of congenital malformations in mammals were described or mentioned. Some of these procedures require much less skill and effort than nutritional methods. They have, with some justification, become very popular since they are useful for many aspects of teratologic research. Yet there is still room for further nutritional experiments in this field. The nutritional methods point to the importance of well-defined chemical substances for embryonic development. Some of these compounds are known to be constituents of enzyme systems which may be rendered ineffective by the lack of such nutritional elements. Since it is generally assumed that genetic mechanisms are mediated through enzymatic activities, it is possible that nutritional teratology will eventually benefit genetic investigations concerned with the mode of action of abnormal genes.[32] Similar enzyme systems may be disturbed by genic deficiencies and certain nutritional deficiencies. Developmental geneticists and nutritionists are probably working in close proximity in the area of teratology.

SUMMARY

Among the many methods now available which permit production of congenital malformations in mammals, nutritional experiments hold a place of their own. Such experiments had and have definite value in teratologic research, but caution must be exercised in the application of the results to human conditions, since experimental animals are subjected to artificial environmental conditions which are not likely to be encountered by man.

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